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(54) Title: METHODS FOR ANALYZING LTC₄ SYNTHASE POLYMORPHISMS AND DIAGNOSTIC USE**(57) Abstract**

This invention relates to single nucleotide polymorphisms in the LTC₄ synthase gene, EMBL accession no. U50136, particularly at one or more of positions 375, 815, 1003, 2169 and 2742. The invention also relates to methods and materials for analysing allelic variation in the LTC₄ synthase gene, and to the use of LTC₄ synthase polymorphism in the diagnosis and treatment of leukotriene mediated diseases such as asthma and allergic rhinitis.

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METHODS FOR ANALYZING LTC₄ SYNTHASE POLYMORPHISMS AND DIAGNOSTIC USE

This invention relates to polymorphisms in the LTC₄ synthase gene. The invention also relates to methods and materials for analysing allelic variation in the LTC₄ synthase gene, and to the use of LTC₄ synthase polymorphism in the diagnosis and treatment of leukotriene mediated diseases such as asthma and allergic rhinitis.

The cysteinyl-leukotrienes, LTC₄, LTD₄ and LTE₄, are potent bronchoconstrictors, increase vascular permeability and increase mucus production in airways. They are implicated in the pathophysiology of asthma and allergic rhinitis and are found at elevated levels in bronchoalveolar lavage from asthma patients, particularly after allergen challenge. LTD₄ and LTE₄ may also enhance the neurogenic inflammatory response in airways. Compounds which inhibit leukotriene synthesis *e.g.* the 5-lipoxygenase inhibitor, zileuton, or the leukotriene receptor antagonist, zafirlukast, have been shown to be effective against asthma and rhinitis (Busse W.W, Clin. Exp. Allergy, 26, 868-879, 1996; see particularly Figure 1 therein which shows the arachidonic acid cascade, indicating the role of LTC₄ synthase in catalysing the formation of LTC₄).

Leukotrienes are derived from membrane phospholipids. Arachidonic acid is released from the phospholipid by cytosolic phospholipase A2 and converted to LTA₄ by 5-lipoxygenase in the presence of 5-lipoxygenase activating protein, FLAP. Polymorphisms in 5-LO have been reported in international patent application WO 97/42347, Brigham & Women's Hospital. LTA₄ is conjugated with reduced glutathione by LTC₄ synthase to form LTC₄. The biologically active metabolites, LTD₄ and LTE₄ are formed, following carrier mediated export of LTC₄, by the sequential action of gamma-glutamyl transpeptidase and dipeptidases.

The LTC₄ synthase gene has been cloned and published as a 4,465 nucleotide genomic sequence comprising 1,446 nucleotides of sequence 5' to the coding sequence, the 5 exons and intervening introns and 3' sequence extending 398 nucleotide beyond the polyA signal (Penrose *et al.*, J. Biol. Chem., 271, 11356-11361, 1996; EMBL accession no. U50136).

One approach is to use knowledge of polymorphisms to help identify patients most suited to therapy with particular pharmaceutical agents (this is often termed "pharmacogenetics"). Pharmacogenetics can also be used in pharmaceutical research to assist the drug selection process. Polymorphisms are used in mapping the human genome and to

elucidate the genetic component of diseases. The reader is directed to the following references for background details on pharmacogenetics and other uses of polymorphism detection: Linder *et al.* (1997), *Clinical Chemistry*, 43, 254; Marshall (1997), *Nature Biotechnology*, 15, 1249; International Patent Application WO 97/40462, Spectra Biomedical; 5 and Schafer *et al.* (1998), *Nature Biotechnology*, 16, 33.

Clinical trials have shown that patient response to treatment with leukotriene antagonists is heterogeneous. Thus there is a need for improved approaches to pharmaceutical agent design and therapy with leukotriene antagonists.

The present invention is based on the discovery of five single nucleotide 10 polymorphisms (SNPs) in the LTC₄ synthase gene. Three SNPs have been found in the 5' untranslated region of the gene and two in the first intron, located at positions 375, 815, 1003, 2169 and 2742 respectively, based on the numbering of U50136. Before our first filing date, we believe there has been no disclosure of polymorphism/allelic variation in the LTC₄ synthase gene.

15 According to one aspect of the present invention there is provided a method for the diagnosis of a single nucleotide polymorphism in LTC₄ synthase in a human, which method comprises determining the sequence of the nucleic acid of the human at one or more of positions 375, 815, 1003, 2169 and 2742 in the LTC₄ synthase gene as defined by the positions in SEQ ID NO: 1, and determining the status of the human by reference to 20 polymorphism in the LTC₄ synthase gene.

The term human includes both a human having or suspected of having a leukotriene mediated disease and an asymptomatic human who may be tested for predisposition or susceptibility to leukotriene mediated disease. At each position the human may be homozygous for an allele or the human may be a heterozygote.

25 In one embodiment of the invention preferably the method for diagnosis described herein is one in which the single nucleotide polymorphism at position 375 is presence of G and/or A.

In another embodiment of the invention preferably the method for diagnosis described herein is one in which the single nucleotide polymorphism at position 815 is presence of C 30 and/or A.

In another embodiment of the invention preferably the method for diagnosis described herein is one in which the single nucleotide polymorphism at position 1003 is presence of A

and/or C. Testing for the presence of the C allele at this position is especially preferred because, without wishing to be bound by theoretical considerations, of its association with increased levels of LTC₄ synthase (as explained herein).

In another embodiment of the invention preferably the method for diagnosis described
5 herein is one in which the single nucleotide polymorphism at position 2169 is presence of C and/or T.

In another embodiment of the invention preferably the method for diagnosis described herein is one in which the single nucleotide polymorphism at position 2742 is presence of C and/or T.

10 The method for diagnosis is preferably one in which the sequence is determined by a method selected from amplification refractory mutation system and restriction fragment length polymorphism.

In another aspect of the invention we provide a method for the diagnosis of leukotriene mediated disease, which method comprises:

- 15 i) obtaining sample nucleic acid from an individual,
ii) detecting the presence or absence of a variant nucleotide at one or more of positions 375, 815, 1003 and 2169 in the LTC₄ synthase gene and
iii) determining the status of the individual by reference to polymorphism in the LTC₄ synthase gene.

20 The published sequence of the LTC₄ synthase gene, EMBL accession number U50136, is shown in SEQ ID NO: 1 in which the variant sites discovered in the present invention are at positions 375, 815, 1003, 2169 and 2742.

Allelic variation at position 375 consists of a single base substitution from G (the published base), for example to A. Allelic variation at position 815 consists of a single base
25 substitution from C (the published base), for example to A. Allelic variation at position 1003 consists of a single base substitution from A (the published base), for example to C. Allelic variation at position 2169 consists of a single base substitution from C (the published base), for example to T. Allelic variation at position 2742 consists of a single base substitution from C (the published base), for example to T. The status of the individual may be determined by
30 reference to allelic variation at one, two, three, four or all five of the above loci.

Sanak *et al.* (1998), *Lancet*, **350**, 1599, have reported an increased risk of aspirin induced asthma (AIA) being associated with the polymorphism at position 1003. This work

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suggests that the presence of the C allele at position 1003 leads to increased levels of LTC₄ synthase (see also Cowburn *et al.* (1998), J. Clin. Invest., **101**, 834). AIA affects about 10 % of adult asthmatics. Aspirin and other cyclo-oxygenase inhibitors cause release of LTs into airways, leading to an asthma attack in susceptible individuals. Clinical approaches to deal
5 with AIA include pretreatment with anti-leukotriene drugs (Szczeklik (1997), Allergy, **52**, 613-9). Commentators have written approvingly of the clinical utility of detection of LTC₄ polymorphisms (Holgate (1998), Lancet, **351**, 1300-1301, see last paragraph in particular). Anti-leukotriene drugs have been reviewed in the following publications: Horwitz *et al.* (1998), Am J Respir Crit Care Med, **157**, 1363 (see particularly Table 1 for a list of drugs);
10 and Tan (1998), Current Opinion in Pulmonary Medicine, **4**, 25.

The test sample of nucleic acid is conveniently a sample of blood, bronchoalveolar lavage fluid, sputum, or other body fluid or tissue obtained from an individual. It will be appreciated that the test sample may equally be a nucleic acid sequence corresponding to the sequence in the test sample, that is to say that all or a part of the region in the sample nucleic
15 acid may firstly be amplified using any convenient technique e.g. PCR, before use in the analysis of LTC₄ synthase variation.

It will be apparent to the person skilled in the art that there are a large number of analytical procedures which may be used to detect the presence or absence of variant nucleotides at one or more of positions 375, 815, 1003, 2169 and 2742 in the LTC₄ synthase
20 gene. In general, the detection of allelic variation requires a mutation discrimination technique, optionally an amplification reaction and a signal generation system. Table 1 lists a number of mutation detection techniques, some based on the PCR. These may be used in combination with a number of signal generation systems, a selection of which is listed in Table 2. Further amplification techniques are listed in Table 3. Many current methods for the
25 detection of allelic variation are reviewed by Nollau *et al.*, Clin. Chem. **43**, 1114-1120, 1997; and in standard textbooks, for example "Laboratory Protocols for Mutation Detection", Ed. by U. Landegren, Oxford University Press, 1996 and "PCR", 2nd Edition by Newton & Graham, BIOS Scientific Publishers Limited, 1997.

Abbreviations:

AIA	Aspirin induced asthma
ALEX™	Amplification refractory mutation system linear extension
APEX	Arrayed primer extension
ARMST™	Amplification refractory mutation system
b-DNA	Branched DNA
CMC	Chemical mismatch cleavage
bp	base pair
COPS	Competitive oligonucleotide priming system
DGGE	Denaturing gradient gel electrophoresis
FLAP	5-lipoxygenase activating protein
FRET	Fluorescence resonance energy transfer
LCR	Ligase chain reaction
5-LO	5-Lipoxygenase
LT	Leukotriene
MASDA	Multiple allele specific diagnostic assay
NASBA	Nucleic acid sequence based amplification
OLA	Oligonucleotide ligation assay
PCR	Polymerase chain reaction
PTT	Protein truncation test
RFLP	Restriction fragment length polymorphism
SDA	Strand displacement amplification
SNP	Single nucleotide polymorphism
SSCP	Single-strand conformation polymorphism analysis
SSR	Self sustained replication
TGGE	Temperature gradient gel electrophoresis

Table 1 - Mutation Detection Techniques**General:** DNA sequencing, Sequencing by hybridisation

5 **Scanning:** PTT*, SSCP, DGGE, TGGE, Cleavase, Heteroduplex analysis, CMC, Enzymatic mismatch cleavage

* Note: not useful for detection of promoter polymorphisms.

Hybridisation Based

Solid phase hybridisation: Dot blots, MASDA, Reverse dot blots, Oligonucleotide
5 arrays (DNA Chips)

Solution phase hybridisation: Taqman™ - US-5210015 & US-5487972 (Hoffmann-La Roche), Molecular Beacons - Tyagi *et al* (1996), Nature Biotechnology, **14**, 303; WO 95/13399 (Public Health Inst., New York)

Extension Based: ARMST™, ALEX™ - European Patent No. EP 332435 B1 (Zeneca
10 Limited), COPS - Gibbs *et al* (1989), Nucleic Acids Research, **17**, 2347.

Incorporation Based: Mini-sequencing, APEX

Restriction Enzyme Based: RFLP, Restriction site generating PCR

Ligation Based: OLA

Other: Invader assay

15

Table 2 - Signal Generation or Detection Systems

Fluorescence: FRET, Fluorescence quenching, Fluorescence polarisation - United Kingdom Patent No. 2228998 (Zeneca Limited)

Other: Chemiluminescence, Electrochemiluminescence, Raman, Radioactivity, Colorimetric,
20 Hybridisation protection assay, Mass spectrometry

Table 3 - Further Amplification Methods

SSR, NASBA, LCR, SDA, b-DNA

25 Preferred mutation detection techniques include ARMST™, ALEX™, COPS, Taqman, Molecular Beacons, RFLP, and restriction site based PCR and FRET techniques.

Particularly preferred methods include ARMST™ and RFLP based methods. ARMST™ is an especially preferred method.

In a further aspect, the diagnostic methods of the invention are used to assess the
30 efficacy of therapeutic compounds in the treatment of asthma, rhinitis and other leukotriene mediated diseases. The polymorphisms identified in the present invention occur in the 5' untranslated region and the first intron of the LTC₄ synthase gene, regions which are of

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importance in the control of gene transcription and gene translation. Furthermore, each of the variant positions is located within a known transcription factor binding site; it is believed that substitution of A at variant position 375 modifies an AP-2 CS4 transcription factor binding site, substitution of A at variant position 815 modifies an AP-2 CS5 transcription factor binding site, substitution of C at variant position 1003 modifies the glucocorticoid receptor binding site GGGACA and substitution of T at variant position 2169 disrupts an MREc-(3) transcription factor binding site.

Example 3 below describes another polymorphism which is substitution of T for C at position 2742. This variant disrupts a RIPE3b site (Shieh and Tsai, J. Biol. Chem. **266**, 16708-16714, 1991).

Assays, for example reporter-based assays, may be devised to detect whether one or more of the above polymorphisms affect transcription levels and/or message stability.

Individuals who carry particular allelic variants of the LTC₄ synthase gene may therefore exhibit differences in their ability to regulate enzyme biosynthesis under different physiological conditions and will display altered abilities to react to different diseases. In addition, differences in enzyme regulation arising as a result of allelic variation may have a direct effect on the response of an individual to drug therapy. LTC₄ synthase polymorphism may therefore have the greatest effect on the efficacy of drugs designed to modulate the activity of LTC₄ synthase or other components of the leukotriene pathway. However, the polymorphisms may also affect the response to agents acting on other biochemical pathways regulated by leukotrienes. The diagnostic methods of the invention may therefore be useful both to predict the clinical response to such agents and to determine therapeutic dose.

In a further aspect, the diagnostic methods of the invention, are used to assess the predisposition and/or susceptibility of an individual to diseases mediated by leukotrienes. LTC₄ synthase polymorphism may be particularly relevant in the development of asthma and other inflammatory diseases such as allergic rhinitis and the present invention may be used to recognise individuals who are particularly at risk from developing these conditions.

In a further aspect, the diagnostic methods of the invention are used in the development of new drug therapies which selectively target one or more allelic variants of the LTC₄ synthase gene. Identification of a link between a particular allelic variant and predisposition to disease development or response to drug therapy may have a significant

impact on the design of new drugs. Drugs may be designed to regulate the biological activity of variants implicated in the disease process whilst minimising effects on other variants.

In a further diagnostic aspect of the invention the presence or absence of variant nucleotides is detected by reference to the loss or gain of sites recognised by restriction enzymes. In the accompanying Example 1 we provide details of convenient sites that are lost or gained as a result of LTC₄ synthase gene polymorphisms. The person of ordinary skill will be able to design and implement diagnostic procedures based on the detection of restriction fragment length polymorphism due to the loss or gain of one or more of the sites listed in Examples 1 or 3.

10 In yet a further aspect the invention provides a variant of the LTC₄ synthase gene comprising one or more of the specific polymorphisms at positions 375, 815, 1003 and 2169.

Further aspects of this invention comprise the 5' untranslated region of the LTC₄ synthase gene comprising a polymorphism at one or more of positions 375, 815 and 1003. In particular the polymorphism at position 375 is G to A. In particular the polymorphism at
15 position 815 is C to A. In particular the polymorphism at position 1003 is A to C. Another aspect of this invention comprises the first intron of the LTC₄ synthase gene comprising a polymorphism at position 2169; in particular this polymorphism is C to T.

According to another aspect of the present invention there is provided a nucleic acid comprising the 5' untranslated region of LTC₄ synthase comprising a polymorphism
20 corresponding with one or more of positions 375, 815 and 1003 as defined by the positions in SEQ ID NO: 1 and in which there is an A at position 375, an A at position 815 and a C at position 1003. The 5' untranslated region of LTC₄ synthase is defined as positions 1-1446 of SEQ ID NO: 1. Fragments of the 5' untranslated region comprising at least one of these allelic variants are also within the scope of the invention.

25 Fragments are at least 17 bases, more preferably at least 20 bases, more preferably at least 30 bases. Complementary strands are also within the scope of the invention.

According to another aspect of the present invention there is provided a nucleic acid comprising the first intron of the LTC₄ synthase gene comprising a polymorphism at one or more of positions 2169 and 2742 as defined by the position in SEQ ID NO: 1 and in which
30 there is a T at position 2169 and there is a T at position 2742. The first intron of the LTC₄ synthase gene is defined as positions 1505-2949 of SEQ ID NO: 1. Fragments of the first

intron comprising at least one of these allelic variants are also within the scope of the invention.

The invention further provides nucleotide primers which detect the LTC₄ synthase gene polymorphisms of the invention.

5 According to another aspect of the present invention there is provided a diagnostic nucleic acid primer capable of detecting a LTC₄ synthase gene polymorphism at one or more of positions 375, 815, 1003, 2169 and 2742 in the LTC₄ synthase gene as defined by the positions in SEQ ID NO: 1.

A diagnostic nucleic acid primer is defined as an allele specific primer, used, generally
10 together with a constant primer, in an amplification reaction such as a PCR reaction, which provides the discrimination between alleles through selective amplification of one allele at a particular sequence position e.g. as used for ARMSTM assays, see Example 2 herein. The diagnostic primer is preferably 17- 50 nucleotides, more preferably about 17-35 nucleotides, more preferably about 17-30 nucleotides.

15 We provide diagnostic primers comprising the sequences set out below as well as derivatives thereof wherein about 6-8 of the nucleotides at the 3' terminus are identical to the sequences given below and wherein up to 10, such as up to 8, 6, 4, 2, or 1 of the remaining nucleotides may be varied without significantly affecting the properties of the diagnostic primer. Conveniently, the sequence of the diagnostic primer is as written below, or more
20 preferably as described in Example 2 below. The diagnostic primer is preferably 17- 50 nucleotides, more preferably about 17-35 nucleotides, more preferably about 17-30 nucleotides.

Primer number*	Allelic variant detected	Diagnostic (Allele Specific) Primer sequence
1	375 A	GGGGCGGCCCGGGGCGCTCCAGGCGGGCA
2	815 A	CTTGGACAGGTTTCCTCCTGGCAGGGTGA
3	1003 C	GGGTTGCCAGGAACAGCCTGGATGGGGACC
4	2169 T	ATGGTCCGACGGGAGGTCTGGGGAGGGAGT
5	375 A	CTCCTGCCTGGAGTTCTGGGTGTCTCCCTT
6	815 A	TAGTCGTTGTAGGGGTTCATGCACAAGGT
7	1003 C	TAATCCTCCACCCACCTTATCTGTTCCTCG
8	2169 T	GACCACACACAGACCAGTGCTGGCTGTGCA

* Primers 1-8 are represented as SEQ ID NO: 2-9 respectively.

The primers may be manufactured using any convenient method of synthesis. Examples of such methods may be found in standard textbooks, for example "Protocols for Oligonucleotides and Analogues; Synthesis and Properties," Methods in Molecular Biology Series; Volume 20; Ed. Sudhir Agrawal, Humana ISBN: 0-89603-247-7; 1993; 1st Edition. If
5 required the primer(s) may be labelled to facilitate detection.

According to another aspect of the present invention there is provided an allele-specific oligonucleotide probe capable of detecting a LTC₄ synthase gene polymorphism at one or more of positions 375, 815, 1003, 2169 and 2742 in the LTC₄ synthase gene as defined by the positions in SEQ ID NO: 1.

10 The allele-specific oligonucleotide probe is preferably 17- 50 nucleotides, more preferably about 17-35 nucleotides, more preferably about 17-30 nucleotides.

The design of such probes will be apparent to the molecular biologist of ordinary skill. Such probes are of any convenient length such as up to 50 bases, up to 40 bases, more conveniently up to 30 bases in length, such as for example 8-25 or 8-15 bases in length. In
15 general such probes will comprise base sequences entirely complementary to the corresponding wild type or variant locus in the LTC₄ gene. However, if required one or more mismatches may be introduced, provided that the discriminatory power of the oligonucleotide probe is not unduly affected. The probes of the invention may carry one or more labels to facilitate detection.

20 According to another aspect of the present invention there is provided a diagnostic kit comprising a diagnostic primer of the invention and/or an allele-specific oligonucleotide primer of the invention.

The diagnostic kits may comprise appropriate packaging and instructions for use in the methods of the invention. Such kits may further comprise appropriate buffer(s) and
25 polymerase(s) such as thermostable polymerases, for example taq polymerase.

The LTC₄ synthase gene has been mapped to chromosome 5q35 (Penrose *et al*, J. Biol. Chem. 271, 11356-11361, 1996). In another aspect of the invention, the single nucleotide polymorphisms of this invention may be used as genetic markers for this region in linkage studies. This particularly applies to the polymorphism at 1003 because of its relatively high
30 frequency, (Krugylak, Nature Genetics, 17, 21-24, 1997).

According to another aspect of the present invention there is provided a method of treating a human in need of treatment with an antileukotriene drug in which the method comprises:

- i) diagnosis of a single nucleotide polymorphism in LTC₄ synthase in the human, which
5 diagnosis comprises determining the sequence of the nucleic acid at one or more of positions 375, 815, 1003, 2169 and 2742 in the LTC₄ synthase gene as defined by the positions in SEQ ID NO: 1, and determining the status of the human by reference to polymorphism in the LTC₄ synthase gene; and
 - ii) administering an effective amount of an antileukotriene drug.
- 10 Preferably determination of the status of the human is clinically useful. Examples of clinical usefulness include deciding which antileukotriene drug or drugs to administer and/or in deciding on the effective amount of the drug or drugs.

According to another aspect of the present invention there is provided use of an antileukotriene drug in preparation of a medicament for treating a leukotriene mediated
15 disease in a human diagnosed as having a single nucleotide polymorphism at one or more of positions 375, 815, 1003, 2169 and 2742 in LTC₄ synthase gene as defined by the positions in SEQ ID NO: 1.

According to another aspect of the present invention there is provided a pharmaceutical pack comprising an antileukotriene drug and instructions for administration of
20 the drug to humans diagnostically tested for a single nucleotide polymorphism at one or more of positions 375, 815, 1003, 2169 and 2742 in LTC₄ synthase gene as defined by the positions in SEQ ID NO: 1.

Suitable antileukotriene drugs include leukotriene D₄ receptor antagonists, FLAP antagonists and 5-lipoxygenase inhibitors (see particularly Table 1 in the following
25 publication for a list of drugs, Horwitz *et al.* (1998), Am J Respir Crit Care Med, 157, 1363), preferably leukotriene D₄ receptor antagonists, more preferably montelukast and zafirlukast, and of these zafirlukast is most preferred.

Testing for the presence of the C allele at position 1003 is especially preferred because, without wishing to be bound by theoretical considerations, of its association with
30 increased levels of LTC₄ synthase (as explained herein).

The invention will now be illustrated but not limited by reference to the following Examples. All temperatures are in degrees Celsius.

In the Examples below, unless otherwise stated, the following methodology and materials have been applied.

AMPLITAQ™, available from Perkin-Elmer Cetus, is used as the source of thermostable DNA polymerase.

- 5 General molecular biology procedures can be followed from any of the methods described in "Molecular Cloning - A Laboratory Manual" Second Edition, Sambrook, Fritsch and Maniatis (Cold Spring Harbor Laboratory, 1989).

Electropherograms were obtained in a standard manner: data was collected by ABI377 data collection software and the wave form generated by ABI Prism sequencing analysis
10 (2.1.2).

Example 1

Identification of Polymorphisms

1. Methods

15 DNA Preparation

DNA was prepared from frozen blood samples collected in EDTA following protocol I (Molecular Cloning: A Laboratory Manual, p392, Sambrook, Fritsch and Maniatis, 2nd Edition, Cold Spring Harbor Press, 1989) with the following modifications. The thawed blood was diluted in an equal volume of standard saline citrate instead of phosphate buffered
20 saline to remove lysed red blood cells. Samples were extracted with phenol, then phenol/chloroform and then chloroform rather than with three phenol extractions. The DNA was dissolved in deionised water.

Template Preparation

Templates were prepared by PCR using the oligonucleotide primers and annealing
25 temperatures set out below. The extension temperature was 72° and denaturation temperature 94°. Generally 50 ng of genomic DNA was used in each reaction and subjected to 40 cycles of PCR.

Fragment	Forward Oligonucleotide	Reverse Oligonucleotide	Annealing Temperature	Time	% DMSO
62-1043	62-87	1021-1043	62°	60 s	5

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271-407	271-291	388-407	60°	45 s	0
417-1043	417-437	1021-1043	60°	45 s	10
851-1824	851-874	1801-1824	62°	60 s	5
1503-2400	1503-1524	2379-2400	65°	60 s	10

For dye-primer sequencing these primers were modified to include T7 and SP6 primer sequences (ABI protocol P/N 402114, Applied Biosystems) at the 5' end of the forward and reverse oligonucleotides respectively.

5 Chemical Mismatch Cleavage (CMC)

CMC was carried out as described by Rowley *et al.* (Genomics 30, 574-582, 1995) using internal labelling of probe and target with fluorescent dyes (RG6 or R110). 6 % Acrylamide gels were run on an automated DNA sequencer (ABI 377, Applied Biosystems) on 12 cm plates (under module GS12-2400A) and analysed with suitable software (ABI

10 GeneScan™ 2.1).

Dye Primer Sequencing

Dye-primer sequencing using T7 and SP6 primers was as described in the ABI protocol P/N 402114 for the ABI Prism™ dye primer cycle sequencing core kit with "AmpliTaq FS"™ DNA polymerase, modified in that the annealing temperature was 45° and

15 DMSO was added to the cycle sequencing mix to a final concentration of 5 %.

The extension reactions for each base were pooled, ethanol/sodium acetate precipitated, washed and resuspended in formamide loading buffer.

4.25 % Acrylamide gels were run on an automated sequencer (ABI 377, Applied Biosystems).

20

2. Results

All positions are based on the U50136 numbering.

Variant Position 375

CMC analysis of fragment 1 (62-1043) produced cleavage products of approximately
25 300 bp and 670 bp in 9/49 subjects. Dye-primer sequence analysis of fragment 1 from 2 subjects showing this pattern revealed a substitution of A for G at position 375. This was

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confirmed by sequencing 6 clones of fragment 1 from one of these subjects; 5/6 had A at position 375 and 1/6 had G.

Substituting A for G at position 375 modifies a Mnl I site at position 368.

PCR products from LTC₄ synthase position 271 to 407 from 49 subjects were digested with 5 Mnl I. This product contains an invariant Mnl I site at position 335 giving an invariant 61 bp fragment and a polymorphic fragment, 72 bp in the absence of site 368 or 33 and 39 base pairs if the Mnl I site at 368 is present. 9/49 subjects gave both the 72 bp and 33/39 bp products indicating that the Mnl I site at position 368 was lost from one allele and these subjects were heterozygous at position 375. The frequency of the A allele at 375 is thus 9/98.

10 Additional RFLPs generated by this variant are loss of an M.CviA IV and M.Sss I site. This variant modifies a transcription factor binding site AP-2 CS4.

Variant Position 815

CMC analysis of fragment 1 produced cleavage products of approximately 230 bp and 750 bp in 2/49 subjects. Dye-primer sequence analysis of fragment 1 from 1 subject showing 15 this pattern revealed a substitution of A for C at position 815. This was confirmed by sequencing 8 clones of fragment 1 from this subject. 4/8 had A at position 815 and 4/8 had C.

Substituting A for C at position 815 generates an Ava II site at 813.

PCR products from LTC₄ synthase position 417 to 1043 from 53 subjects were digested with Ava II. In 5/53 subjects an Ava II site at position 815 was created. These 20 subjects were heterozygous at position 815. The frequency of the A allele was thus 5/106 alleles.

Additional RFLPs generated by this variant are loss of Aca I, CviK I, M.CviA IV, CviJ I and Hae III sites and gain of Asp697 I, VpaK11A I and Sin I sites.

This variant modifies a transcription factor binding site: AP-2 CS5.

25 Variant Position 1003

CMC analysis of fragment 1 produced a cleavage product of approximately 940 bp in 22/40 subjects. CMC analysis of fragment 2 (851-1824) produced a band of approximately 800 bp. Dye-primer sequence analysis of fragment 2 from 24 subjects showing this pattern revealed a substitution of C for A at position 1003. This was confirmed by sequencing 14 30 clones of fragment 1 from 2 subjects with the 940 bp cleavage product. 6/14 had C at position 1003 and 8/14 had A.

Substituting C for A at position 1003 generates an Ava II site at position 999.

-15-

PCR products from LTC₄ synthase position 417 to 1043 from 53 subjects were digested with Ava II. In 26/53 subjects an Ava II site at position 1003 was created. One of these subjects was homozygous C/C at position 815 and 25 were heterozygous C/A. The frequency of the C allele was thus 27/106 alleles.

5 The 1003 C variant is not on the same chromosome as the 815 A variant.

Additional RFLPs generated by this variant are gain of sites for Bcr I, AhaB I, Asp697 I, VpaK11A I, Asu I, Fmu I, Sau96 I, Sin I, Nla IV, Asp I, Asp748 I, BsaC I, Dsa V, Eco1831 I, Hin2 I, Hpa II, Msp I, Bcn I, Nci I, ScrF I and M.Sss I.

This variant modifies the glucocorticoid receptor binding site GGGACA, (Chan *et al.*,
10 J. Biol. Chem. **266**, 22634-22644, 1991).

Sanak *et al.* (1998), Lancet, **350**, 1599, have reported an increased risk of aspirin induced asthma (AIA) being associated with this polymorphism (Sanak's position -444 is equivalent to our position 1003). AIA affects about 10 % of adult asthmatics. Aspirin and other cyclo-oxygenase inhibitors cause release of LTs into airways, leading to an asthma
15 attack. Clinical approaches to deal with AIA include pretreatment with anti-leukotriene drugs (Szczeklik (1997), Allergy, **52**, 613-9). Commentators have written approvingly of the clinical utility of detection of LTC₄ polymorphisms (Holgate (1998), Lancet, **351**, 1300-1301, see last paragraph in particular).

Variant Position 2169

20 Dye-primer sequencing of fragment 6 (1503-2400) from 47 subjects demonstrated a substitution of T for C at position 2169 in 3 subjects.

Substituting T for C at position 2169 generates an Apa LI site at position 647.

Fragment 6 was digested with ApaL I. In 3/54 subjects an Apa LI site was created.

These subjects were heterozygous for the RFLP, thus the frequency of the T allele at position
25 2169 is 3/108 alleles.

Additional RFLPs generated by this variant are loss of M.CviA IV, Bca I, Hinp I, Hinp1 I, Cfo I, Hha I and M.Sss I sites and gain of Aaq I, Bka1125 I, BsaG I, CviR I, BsiHKA I, Bsp 1286 I, Hgi A I and Nsp II sites.

This variant disrupts an MREc-(3) site (Labbe *et al.*, Nuc. Acid Res. **19**, 4225-4231
30 1991).

Example 2**Detection of Variants 375, 815, 1003 and 2169 using ARMS™.**

The following primers were used in ARMS™ PCR to distinguish allelic variants at positions 375, 815, 1003 and 2169 of the LTC₄ synthase gene.

5

Allelic Variant Detected	Allele Specific Primer Sequence**	Constant Primer Sequence***
375 G A	GGAGTTCTGGGTGTCTCCATC GGAGTTCTGGGTGTCTCCATT	GGTCAGTCTGGACTTTGCCAC
815 C C A A	TAGGGGTTCCATGCACAAGGG TAGGGGTTCCATGCACAATGG TAGGGGTTCCATGCACAAGGT TAGGGGTTCCATGCACAATGT	TTGTTACCTTGAGGCAAGAGG
1003 A C A C	CACCCACCTTATCTGTTCCT CACCCACCTTATCTGTTCCTCG GGAACAGCCTGGATGGGGTCA GGAACAGCCTGGATGGGGTCC	AGGCTGGCAGGCATGAGGTTT TTCGTGCCCTTCCTTGCCTA
2169 C T	CAGACCAGTGCTGGCTGTACG CAGACCAGTGCTGGCTGTACA	CTCCAGCTGCTCCTGCACTGA

** These primers are represented by SEQ ID NO: 10-21 respectively.

*** These primers are represented by SEQ ID NO: 22-26 respectively.

10

Genomic DNA (50 ng) was amplified for 35 cycles with the above pairs of primers. The annealing temperatures were 62°, 60°, 64° and 60° for 375, 815, 1003 and 2169 respectively.

Homozygotes for the less common allele were only available for position 1003. The above primers and conditions would distinguish A/A, A/C and C/C genotypes at position 1003.

375 A/A homozygotes were not available so it could not be demonstrated that the G specific primer would not recognise A/A homozygotes but the A specific primer did not

-17-

recognise G/G homozygotes. 815 A/A homozygotes were not available so it could not be demonstrated that the C specific primer would not recognise A/A homozygotes but the A specific primer did not recognise C/C homozygotes. 2169 T/T homozygotes were not available so it could not be demonstrated that the C specific primer would not recognise T/T homozygotes but the T specific primer did not recognise C/C homozygotes.

Example 3

Polymorphism at Position 2742

Dye primer sequencing, as described in Example 1, of fragment 2180-2972 from 510 subjects demonstrated a substitution of T for C at position 2742 in 2 subjects. Template was prepared as described in Example 1 using the conditions set out below.

Fragment	Forward Oligonucleotide	Reverse Oligonucleotide	Annealing Temperature	Time	% DMSO
2180-2973	2180-2200	2953-2973	65°	60 s	10

This variant disrupts a RIPE3b site (Shieh and Tsai, J. Biol. Chem. 266, 16708-16714, 1991).

CLAIMS

1. A method for the diagnosis of a single nucleotide polymorphism in LTC₄ synthase in a human, which method comprises determining the sequence of the nucleic acid of the human at
5 one or more of positions 375, 815, 1003, 2169 and 2742 in the LTC₄ synthase gene as defined by the positions in SEQ ID NO: 1, and determining the status of the human by reference to polymorphism in the LTC₄ synthase gene.
2. A method according to claim 1 in which the single nucleotide polymorphism at position 375 is presence of G and/or A.
- 10 3. A method according to claim 1 in which the single nucleotide polymorphism at position 815 is presence of C and/or A.
4. A method according to claim 1 in which the single nucleotide polymorphism at position 1003 is presence of A and/or C.
5. A method according to claim 1 in which the single nucleotide polymorphism at
15 position 2169 is presence of C and/or T.
6. A method according to claim 1 in which the single nucleotide polymorphism at position 2742 is presence of C and/or T.
7. A method according to any one of claims 1-6 in which the sequence is determined by a method selected from amplification refractory mutation system and restriction fragment length
20 polymorphism.
8. A nucleic acid comprising the 5' untranslated region of LTC₄ synthase comprising a polymorphism corresponding with one or more of positions 375, 815 and 1003 as defined by the positions in SEQ ID NO: 1 and in which there is an A at position 375, an A at position 815 and a C at position 1003.
- 25 9. A nucleic acid comprising the first intron of the LTC₄ synthase gene comprising a polymorphism at one or more of positions 2169 and 2742 as defined by the position in SEQ ID NO: 1 and in which there is a T at position 2169 and there is a T at position 2742.
10. A diagnostic nucleic acid primer capable of detecting a LTC₄ synthase gene polymorphism at one or more of positions 375, 815, 1003, 2169 and 2742 in the LTC₄ synthase gene as
30 defined by the positions in SEQ ID NO: 1.

11. An allele-specific oligonucleotide probe capable of detecting a LTC₄ synthase gene polymorphism at one or more of positions 375, 815, 1003, 2169 and 2742 in the LTC₄ synthase gene as defined by the positions in SEQ ID NO: 1.
12. A diagnostic kit comprising a diagnostic primer as defined in claim 10 and/or an
5 allele-specific oligonucleotide primer as defined in claim 11.
13. A method of treating a human in need of treatment with an antileukotriene drug in which the method comprises:
- i) diagnosis of a single nucleotide polymorphism in LTC₄ synthase in the human, which diagnosis comprises determining the sequence of the nucleic acid at one or more of positions
10 375, 815, 1003, 2169 and 2742 in the LTC₄ synthase gene as defined by the positions in SEQ ID NO: 1, and determining the status of the human by reference to polymorphism in the LTC₄ synthase gene; and
 - ii) administering an effective amount of an antileukotriene drug.
14. Use of an antileukotriene drug in preparation of a medicament for treating a leukotriene
15 mediated disease in a human diagnosed as having a single nucleotide polymorphism at one or more of positions 375, 815, 1003, 2169 and 2742 in LTC₄ synthase gene as defined by the positions in SEQ ID NO: 1.

-1-

SEQUENCE LISTING

(1) GENERAL INFORMATION:

5

(i) APPLICANT:

- (A) NAME: Zeneca Limited
 (B) STREET: 15 Stanhope Gate
 (C) CITY: London
 10 (D) STATE: England
 (E) COUNTRY: United Kingdom
 (F) POSTAL CODE (ZIP): W1Y 6LN
 (G) TELEPHONE: 0171 304 5000
 (H) TELEFAX: 0171 304 5151
 15 (I) TELEX: 0171 304 2042

(ii) TITLE OF INVENTION: METHODS

(iii) NUMBER OF SEQUENCES: 26

20

(iv) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
 (B) COMPUTER: IBM PC compatible
 (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 25 (D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

30

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 4465 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

35

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

	GAGCTCACAG AGCCCCCAGC TGGGGCATAT CTGGTTTCCG GGGGCAGGGG CGATACCCAG	60
40	AGGAGGAAGA AGGGATTCTG AGAGAGCCCA ACAGGCTCCG AGCCTCAGGC TGGAGCTGAG	120
	CTTGGGGCAG CAAGGAAGGA CCAGGTGCGA GGGCAGAACC ATGCGGCCCG ACCCCTGCAG	180
	CACGGCCTGT GGCCTCCCCC AGCTCCTGCC CGTGCTTCTG GGTCACTCTG GACTTTGCCA	240
	CTTCTGACCA AAAGCCACCG CAAACCCACT CAAGCCAAAA GAGGAAGTGA CCGTTAGGCC	300
	CAACTGGGAA GGCTGGCGGC CAGGGGCACT CCAGGCAGGG CGAGGGGGGC GGCCGGGGGC	360
45	GCTCCAGGCG GGGCGAGGGA GACACCCAGA ACTCCAGGCA GGAGTCCTCG GGTGCCACCT	420
	TTCCTCTCCA CCTGGCCCTG CGTGGGCTCT GTCCTCAGGG TGGCCCGCCG TAGTCCCCCT	480
	CCCCACTCTG AGTTTCCTGT CCCAAAGTCC TAAGGAAGTT TCCAGAACTA CATCTCACCA	540

	TCTTGAGTCA	GCCTTGGCTC	AGTGTCCATC	TCACAGGCCT	GGAAGGGGCA	GGAGTCAGCA	600
	CTGTCCAGAC	CACAGGGCCT	GAGTGTGGGG	AGGGCAGCCG	TCTAGGAAGG	TGGTGGAGGG	660
	TTGTTACCTT	GAGGCAAGAG	GGCTGCGGGG	CAGAAAGACA	CAGCAGGTGA	CTGTTGTGGG	720
	AGGCCCAAGA	GAGGCCTGGG	AGAGGGATGG	CCCACAAGGG	CTGACCCTCC	CGCCACCCAG	780
5	GGGGCCTTGG	ACAGGTTTCC	TCCTGGCAGG	GTGGCCCTTG	TGCATGGAAC	CCCTACAACG	840
	ACTAAGGCTG	GCAGGCATGA	GGTTTCTCTGA	AGGAGAAAGA	GCTTGTGGGG	CCCAGTGTGG	900
	CTGGGGGGGC	GCTGGGACTC	CATTCTGAAG	CCAAAGGCAC	TGGGAAGGGC	TTCCGCAGAG	960
	GAGGGTTTGG	CAGGGGTTCG	CAGGAACAGC	CTGGATGGGG	ACAGGGAACA	GATAAGGTGG	1020
	GTGGAGGAGT	TAGCCGGGAG	CCTGGGGCTG	GCTCCAGCAT	GATGTGGGGG	TCTGCAAGGC	1080
10	CCTGGAGAAA	GTGGGGTGGT	GCAGCAGGGG	GCACACCCAC	AGCTGGAGCT	GACCCAGATG	1140
	GACAGCTTGG	GCTCTGCCAC	GCGGGACTAG	GCAAGGAAGG	GGCACGAACA	AGCAGGAAGT	1200
	GGTGAGGCGG	TCTCCAGCTA	GCTGCTCTCC	CCTGCCCAGA	CTTTGGTTTC	CTCCCTGCTG	1260
	GCTTGGCCTG	GCTCCCTGGC	TCTGTGTGGT	ATGGTCACAC	CCCCGTGCAC	CCCCTCCACT	1320
	GAGATGGGGC	GGGGAGAGCA	CCGAGGCTGC	TCTTCTCTC	CTGGGCCGTC	CTCTGAGCAG	1380
15	CAGACGGGGC	TAAGCGTTCC	CCAGCTCGCC	TTACACACAC	GCCCCTGCCA	CCACACCGAC	1440
	GGTACCATGA	AGGACGAGGT	AGCTCTACTG	GCTGCTGTCA	CCCTCCTGGG	AGTCCTGCTG	1500
	CAAGGTGGGC	TGGTTCCTAT	CTAGGAAGAG	GGTGGGCCCT	AGATCCCTAC	AGCTTGCCCT	1560
	CTGCCCCCTA	GGCCCAGGTG	GAGGGCAGAG	GTGGGGACTC	CAGCCCAGGC	CCAAGCTGGA	1620
	AGAGGGTGGG	GACTTTCAGG	GAAGTGGGGG	GCACCTGGCT	GTGAGAGCTG	TAGGACTTGG	1680
20	GGGTGGCAAG	GGTGCCAGGA	CAAATGGTAG	GATAGCCATG	GGCTTGGGGA	AGCTGATCTC	1740
	TGCTCTTTCC	AGCTGTCCCC	TCTCTGGGCG	TCCCAGCAAG	CGGCCCCCAT	TCCCTGGCTC	1800
	TGCTTCAAAG	GCACCTCCAT	ACTGGGACCA	CGTGGAGCAG	GGTAGAGGTG	GGACTCCTTC	1860
	CTCCAGCCCC	CTAAAAAGAG	CCTGCTTAAT	GCCTTTCTCA	GACTGGCCCT	AAAGGACACA	1920
	TTCTTTGGCC	AGATATCCTT	GCCACCTAAG	AGACACCACT	ACTCCACAGT	GTGTGGGCTA	1980
25	GGATAAGGCA	CAGCCTGGGG	AGGGGGCTCT	GAAGGGGCTG	AACAGACAGG	CCAGCCTGAC	2040
	CTCCAGCTGC	TCCTGCAGTG	AGCTGGATGG	CCACCCTGTG	ACACCCATCT	GCAGAGGGCC	2100
	CAGAACCAAA	GGTGCCAGGG	CTGCAGGACT	CAGGGGGAGA	TGGTCCGACG	GGAGGTCTGG	2160
	GGAGGGAGCG	CACAGCCAGC	ACTGGTCTGT	GTGTGGTCTG	GCCTGGCCTC	ACCTGACCAA	2220
	GAGAAGGGCT	CCTGCCCACA	GAGAACTTT	AGGGCCAGCC	CACCCTCTGC	AACTACCCCA	2280
30	GCCCTGGGGT	CCTGGGGTTA	GGCTAGGAGA	GTCCCAGCTG	CAACCTCCTG	GGAGCAGGAG	2340
	AGAAGGTGTC	TGTCAGATTT	AGGCCTGGGA	CCGGAATGCA	GGAACAGAGA	AACTGAGGTT	2400
	TGGAGGCACA	GGGACGCAGG	CTTTAGTGAT	CCCGGCCTGA	GGCAGGGTCA	GAGGGCCCTG	2460
	CTGGTGGGCG	CTGGTAGGTG	GGTGACCAGG	GACTGTTAGC	TACAGGGAGT	GTGCTTCCTT	2520
	GCACCTGGGA	GGATGCAGCC	AGCTCTGCCC	TCAGACTCCC	GAGGCACTTC	CTGGCCAGGG	2580
35	ACCTGAAAGC	TGCAATTGCC	TGTGTTTTGA	GAGTGAAATG	ATTGAGAAAC	AAGGACTCAA	2640
	GTGGTCTCTC	TCGCGGAGCA	GGTGTCCCTG	TGCCTGAATC	ACTCACCTC	CCCCATACAC	2700
	TCACAGGTTG	GGACAGGGCC	TCTCTGCGCC	CCAGGCTTCA	GCCCTGCCCT	CCTCGCTGAA	2760
	TGTCAGGGAC	ACAGGGCAGG	CCAGGGATGG	GTGAGACGAG	AGGTCTCCTC	GGGCGGGGAG	2820
	GGGCGGGGGT	TCCGCCTTAG	GGAGGAGAGG	ACACGGCCAA	GTGAAGGGCC	AGATTGCAGG	2880
40	ATCCCTCCCA	CTCCCATCTC	TGGGGCTTCG	GGTGTCCAGA	CCTGACTCCC	GCTCCCCCTC	2940
	CTCCCCCAGC	CTACTTCTCC	CTGCAGGTGA	TCTCGGCGCG	CAGGGCCTTC	CGCGTGTGCG	3000
	CGCCGCTCAC	CACCGGCCCC	CCCGAGTTCG	AGCGCGTCTA	CCGAGCCCAG	TGAGGCGCGG	3060
	CGGGAGGGCG	CGGGGCGGGG	AGCGAGCCCC	AGGCGGGTCC	GGGTGCGCAGG	ACCATCCCCG	3120
	CCGGCGCGCT	CATCCCACCC	GCCCACCGCA	GGGTGAACTG	CAGCGAGTAC	TTCCCGCTGT	3180
45	TCCTCGCCAC	GCTCTGGGTC	GCCGGCATCT	TCTTTCATGA	AGGTGCGGGT	GTGGGGCAGG	3240
	GGCGCACGCG	CTGGACCCCC	GGGACCCGCG	CAGGGCGCTC	ACCAGGCCCC	TGCGTACCTC	3300
	TCGCAGGGGC	GGCGGCCCTG	TGCGGCCTGG	TCTACCTGTT	CGCGCGCCTC	CGCTACTTCC	3360

-3-

AGGGCTACGC GCGCTCCGCG CAGCTCAGGT GAGGGCCGGG CGGGGAGCGG GGCGGGGCCG 3420
 GGGAAAGATC GCGGGCGGGC GGGGCTCCTG GGGAGCGGGA CCGAAGCTGG GGGCGGGCGA 3480
 CGGGCCGGAG CCCAGCGCCT TTGGGGATTC GGTGGGCGAG CCCTGGCGGC GGCCAGAGGA 3540
 AGTCCCCGTG GGGCCAGGGT TCGGCGGGG AAGAAGCGGG CCTCCTCGCG CCACCTCCCC 3600
 5 GCTGACCGCC GCGCGCAGGC TGGCACCCTG GTACGCGAGC GCGCGCGCCC TCTGGCTGCT 3660
 GGTGGCGCTG GCTGCGCTCG GCCTGCTCGC CCACTTCCTC CCGGCCGCGC TCGCGCGCCG 3720
 GCTCCTCGGA CGGCTCCGGA CGCTGTGCGC GTGGGCTGA GACCAAGGCC CCCGGGCCGA 3780
 CGGAGCCGGG AAAGAAGAGC CGGAGCCTCC AGCTGCCCGG GGGAGGGGCG CTCGCTTCCG 3840
 CATCCTAGTC TCTATCATT AAGTTCTAGT GACCGAGACC CGGGCTGCGT TCTCTGGGTC 3900
 10 CGCGGGGGTG GCGCACCCTG GGCTACGGAG CCTGGAGGGG CCCAGCCCGA GTCCGGGCAG 3960
 CCCGGGGCGG GCTTCCTAGT GCGGCGTGA GAGTGGCTGC GAAGGAACGA GCCCTCCCCC 4020
 TGGGGCGGGA CTGGATCCGG TCTTCACCTC CTACCCCACT CCCTACTCAG CCTCGGGGTC 4080
 ACAAGGCCGC CCAGTCCTGC CGGGGTTCAC CCTCCTAGCG CTCAGCGGTC TCCTCACCAG 4140
 TCCCCCTCCT CAGGGGCCTT CCCTCGACTC TCAGCCGCGC CAGTCCCTCG TCCCCTGGCC 4200
 15 TTCACAGCTG AACTAGATA GAGCCTGTGG CTCTCTCCCC AGGTGAGGGC AGGGGTTTTT 4260
 CTTTTGGTCA GCACTGGATC CCCCTCGTTA ACTGTAGGTG TTCAGGGCAG CCCTCCGAGG 4320
 TCCGCAGAGC TGCGGGCACC ATGGGAACGA AGTGAGTCAG TGACAGGCGG TCTCAAGGAA 4380
 ATGTCCAGAA GCCTTGGGGA TCCAGGGGAG GCCCAGAGAA ACAAAGAAGT GACTTTTAGC 4440
 CAAGTATGCA GGAGAAACGG AGGAG 4465

20

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

25

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

GGGGCGGCCG GGGGCGCTCC AGGCGGGGCA

30

(2) INFORMATION FOR SEQ ID NO: 3:

35

(i) SEQUENCE CHARACTERISTICS:

40

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

45 CTTGGACAGG TTTCTCCTG GCAGGGTGA

30

(2) INFORMATION FOR SEQ ID NO: 4:

-4-

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

GGGTTGCCAG GAACAGCCTG GATGGGGACC

30

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

ATGGTCCGAC GGGAGGTCTG GGGAGGGAGT

30

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

CTCCTGCCTG GAGTTCTGGG TGTCTCCCTT

30

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

-5-

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

TAGTCGTTGT AGGGGTTCCA TGCACAAGGT

30

5 (2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30 base pairs

(B) TYPE: nucleic acid

10 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

15

TAACTCCTCC ACCCACCTTA TCTGTTCCCG

30

(2) INFORMATION FOR SEQ ID NO: 9:

20 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

25

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

GACCACACAC AGACCAGTGC TGGCTGTGCA

30

30

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 base pairs

(B) TYPE: nucleic acid

35 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

40 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

GGAGTTCTGG GTGTCTCCAT C

21

(2) INFORMATION FOR SEQ ID NO: 11:

45

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 base pairs

-6-

- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

- 5 (ii) MOLECULE TYPE: other nucleic acid
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

GGAGTTCTGG GTGTCTCCAT T

21

- 10 (2) INFORMATION FOR SEQ ID NO: 12:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 21 base pairs
 - (B) TYPE: nucleic acid
 - 15 (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: other nucleic acid
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

20

TAGGGGTTCC ATGCACAAGG G

21

- (2) INFORMATION FOR SEQ ID NO: 13:

- 25 (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 21 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

30

- (ii) MOLECULE TYPE: other nucleic acid
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

TAGGGGTTCC ATGCACAATG G

21

35

- (2) INFORMATION FOR SEQ ID NO: 14:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 21 base pairs
 - 40 (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: other nucleic acid
- 45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

TAGGGGTTCC ATGCACAAGG T

21

-7-

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

TAGGGGTTCC ATGCACAATG T

21

15 (2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:

- 20 (A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

25

CACCCACCTT ATCTGTTCCT T

21

(2) INFORMATION FOR SEQ ID NO: 17:

30 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

35

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

CACCCACCTT ATCTGTTCCT G

21

40

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:

- 45 (A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

-8-

- (ii) MOLECULE TYPE: other nucleic acid
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

5 GGAACAGCCT GGATGGGGTC A 21

(2) INFORMATION FOR SEQ ID NO: 19:

- (i) SEQUENCE CHARACTERISTICS:
 - 10 (A) LENGTH: 21 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- 15 (ii) MOLECULE TYPE: other nucleic acid
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

GGAACAGCCT GGATGGGGTC C 21

20 (2) INFORMATION FOR SEQ ID NO: 20:

- (i) SEQUENCE CHARACTERISTICS:
 - 25 (A) LENGTH: 21 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: other nucleic acid
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

30 CAGACCACTG CTGGCTGTAC G 21

(2) INFORMATION FOR SEQ ID NO: 21:

- 35 (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 21 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- 40 (ii) MOLECULE TYPE: other nucleic acid
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

CAGACCACTG CTGGCTGTAC A 21

45

(2) INFORMATION FOR SEQ ID NO: 22:

-9-

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 21 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
5 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: other nucleic acid
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

10 GGTCAGTCTG GACTTTGCCA C

21

- (2) INFORMATION FOR SEQ ID NO: 23:

- (i) SEQUENCE CHARACTERISTICS:
15 (A) LENGTH: 21 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: other nucleic acid
20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

TTGTTACCTT GAGGCAAGAG G

21

- 25 (2) INFORMATION FOR SEQ ID NO: 24:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 21 base pairs
 (B) TYPE: nucleic acid
30 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: other nucleic acid
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

35

AGGCTGGCAG GCATGAGGTT T

21

- (2) INFORMATION FOR SEQ ID NO: 25:

- (i) SEQUENCE CHARACTERISTICS:
40 (A) LENGTH: 21 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- 45 (ii) MOLECULE TYPE: other nucleic acid
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

TTCGTGCCCC TTCCTTGCCT A

21

(2) INFORMATION FOR SEQ ID NO: 26:

5

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

10

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

15 CTCCAGCTGC TCCTGCACTG A

21

INTERNATIONAL SEARCH REPORT

Internat / Application No

PCT/GB 98/02468

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 95 33839 A (SEARLE & CO ;CREELY DAVID PAUL (US); HAUSER SCOTT DAVID (US); WELS) 14 December 1995 see abstract see page 11, line 3 - page 12, line 7 see page 20, line 3 - line 33 see page 22, line 8 - page 24, line 3; claims 1,12,18; example 4	1,7,8, 10-14
Y	WO 95 32280 A (BRIGHAM & WOMENS HOSPITAL) 30 November 1995 see abstract see page 5, line 10 - line 16 see page 15, line 25 - page 16, line 1 see page 29, line 7 - page 33, line 10; claims 4,6,11,12,14,20-23,27; examples 1,2,5	1,8,13, 14



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

27 November 1998

Date of mailing of the international search report

11/12/1998

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk

Authorized officer

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 98/02468

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 93 18178 A (PHILADELPHIA CHILDREN HOSPITAL) 16 September 1993 cited in the application see the whole document	1,7, 10-12
Y	BUSSE W W: "The role of leukotrienes in asthma and allergic rhinitis" CLINICAL AND EXPERIMENTAL ALLERGY, vol. 26, 1996, pages 868-879, XP002086061 cited in the application see abstract see page 870, column 1, paragraph 2 - page 871, column 1, paragraph 1 see page 875, column 1, paragraph 1 - page 876, column 2, paragraph 2 see page 877, column 1, paragraph 2 - paragraph 3	13,14
A	PENROSE J F ET AL.: "Molecular cloning of the gene for human leukotriene C4 synthase" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 271, no. 19, 1996, pages 11356-11361, XP002086062 cited in the application * see especially Fig. 1 * see the whole document	
A	WELSCH D J ET AL: "MOLECULAR CLONING AND EXPRESSION OF HUMAN LEUKOTRIENE-C4 SYNTHASE" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 91, October 1994, pages 9745-9749, XP002044917 * see especially Fig. 2 * see the whole document	
A	NEWTON C R ET AL: "ANALYSIS OF ANY POINT MUTATION IN DNA. THE AMPLIFICATION REFRACTORY MUTATION SYSTEM (ARMS)" NUCLEIC ACIDS RESEARCH, vol. 17, no. 7, 11 April 1989, pages 2503-2516, XP000141596 cited in the application see the whole document	
P,X	SANAK M ET AL.: "leukotriene C4 synthase promotor polymorphism and risk of aspirin-induced asthma" THE LANCET, vol. 350, 1997, pages 1599-1600, XP002086063 cited in the application see the whole document	1,4,8, 10,11, 13,14

INTERNATIONAL SEARCH REPORT

International application No.

PCT/GB 98/ 02468

Box I-- Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim 13
is directed to a method of treatment of the human/animal
body, the search has been carried out and based on the alleged
effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such
an extent that no meaningful international Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all
searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment
of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report
covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is
restricted to the invention first mentioned in the claims, it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 98/02468

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9533839 A	14-12-1995	AU 2638695 A	04-01-1996
WO 9532280 A	30-11-1995	NONE	
WO 9318178 A	16-09-1993	NONE	

-1-

SEQUENCE LISTING

(1) GENERAL INFORMATION:

5

(i) APPLICANT:

(A) NAME: Zeneca Limited
 (B) STREET: 15 Stanhope Gate
 (C) CITY: London
 (D) STATE: England
 (E) COUNTRY: United Kingdom
 (F) POSTAL CODE (ZIP): W1V 6LN
 (G) TELEPHONE: 0171 304 5000
 (H) TELEFAX: 0171 304 5151
 (I) TELEX: 0171 304 2042

10

15

(ii) TITLE OF INVENTION: METHODS

(iii) NUMBER OF SEQUENCES: 26

20

(iv) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
 (B) COMPUTER: IBM PC compatible
 (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 (D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

25

(2) INFORMATION FOR SEQ ID NO: 1:

30

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 4465 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

35

(ii) MOLECULE TYPE: DNA (genomic)

(iii) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

	GAATTAACAG AGCCCCAGC TGGGCTAT CTGGTTTCG GGGCAGGGG CATTACCCAG	60
40	AGGAGGAACA AGGATTCTG AGAGAGCCCA ACAGGCTCCG AGCTCAGGC TGAAGCTGAG	120
	CTTGGXNNG CAAGGAAGGA CCAGGTGCGA GGGCAGACC ATGCGGCCCG ACCCTGCG	180
	CAAGGCTCTT GGCCTCCCC AGCTCCTGCC CGTGCTCTG GGTCACTCTG GACTTTCCTA	240
	CTTCTAACCA AAGGCCACCG CAAACCCCT CAAGCCAAA GAGGAGTGA CCGTTAGGCC	300
	CAACTGGGAA GCGTGGGGC CAGGGCACT CCAGGCAGG CGAGGGGGC GGCCTGGGGC	360
45	CTTCAAGCG GCGGAGGGA GACACGAGA ACTCCAGGCA GGAGTCTCG GATCCACCT	420
	TTCTCTCCA CTTGGCCTG CTTGGCTCT GTCTCAGG TGGCCGCGG TATTCCTCT	480
	CTCCTCTCT ATTTCTCTT CCAAGTCC TAAAGAGTT TCCAGACTA CATTCTACA	540

	TTTTRAGTCA GCGTTGGCTC AGTGTCCATC TCACAGGUCT GGAGGGGGCA GGAGTCAGCA	600
	CTTTCACAGC CACAGGCGCT GAGTGTGGCG AGGGTAGCCG TCTAGGAAGG TGGTGGAGGG	660
	TTGTTTACCTT GAGGGLAAGG GCGTGGCGTG CAGCAAGTCA CAGCAGGTGA CTGTJGTGGG	720
	AGGCCCAAGA GAGGCGTGGG AGAGGAGATG CACACAGGCG CTGACCCCTC CGCCACCCAG	780
5	GCGGCTTGG ACAGGTTTCY TCGTGGCGAG GTTGGCTTTC TCGATGGAGC CCGTACAGG	840
	ACTAGGGCTG GCAGGCATGA GGTTCCTGA AGGAGAAAGA GCTTGTGGCG CCGAGTGTGG	900
	CTGGGGGGGC GCTGGGACTC CATTCGAAAG CCAAGAGGAC TGGCAAGGCG TTCCGCGAGG	960
	GAGGGTTTGG CAGGGGTTGC CAGGAACAGC CTGGATGGGG ACAGGAGACA CATAGAGTGG	1020
	GTGGAGGAGT TAGCCGGGAG CCGGGGCTG GCTCCAGCAT GATGTGGAGG TCTGAGGGG	1080
10	CTTGGAGAAA GTGGGTGGT GCAGCAGGGG GCGACCCAC AGCTGGAGCT GAGCAGATG	1140
	GACAGCTTTC GCTTCCTCAC GCGGGATGAG GCAAGGAGGG GGCACGAAAC AGCAGGAGGT	1200
	CGTACAGGCG TCTCAGAGTA CCGTGTGTTC CCGTGGCGGA CTTTGGTTTC CTCCCTGCTG	1260
	GCTTGGCTTG GCTTCTGTTC TCTTGTGTTC ATGCTGAGAG CCGGTGGAGC CCGCTCCACT	1320
	GAGATGGGGC GCGGAGAGGA CCGAGGCTGC TCTTCTCTTC CTGGGCGGTC CTCTGAGCAG	1380
15	CAGACGGGGC TAAGCGTTCC CAGGCTGCGC TTCAACACAC GCGGTGTGCA CCGACCGGAC	1440
	GCTACCTTGA AGGACGAGGT AGCTCTACTG GCTGCTGTCA GCTTGTGGGG AGTCTGCTG	1500
	CAAGGTGGGC TGCTTCTAT CTAGGAAGAG GTTGGGCTTC AGATGCTTAC AGCTTGGGCT	1560
	CTGCCCCCTA GCGCCAGGTG GAGGGCAGAG GTGGGGACTC CAGGCTGAGC CAGAGCTGCA	1620
	AGAGGTGGGG GACTTTCAGG GAAGTGGGGG GCGCTGGGCT GTGAGGAGTG TAGTACTTGG	1680
20	GCTGTGCGAG GCTGCGAGGA CAAATGGTAG GGTAGGCTTG GCGTTGGGGA AGCTGATCTC	1740
	TGCTCTTTTC AGCTGTGCTC TCTGTGCTTC TCGGCGAGAG CGGCCGCCAT TCGCTGGGTC	1800
	TGCTTCAAGG GCACTTCTAT ACTGTGAGCA CCGTGGAGAG GGTAGAGGTG GAGCTCTTTC	1860
	CTCCAGCCCC CTAAAGAGAG CCGCTTCTAT GCTTGTCTCA GAGTGGGCTT AAGGACACA	1920
	TTCTTGGGCT AGATATCTTT GCGACCTTAG ABACAGCACT ACTCCACAGT GTGTGGGCTA	1980
25	GGATAGGGCA CAGGCTGGGG AGGGGGCTCT GAGGGGGCTG AAGAGAGAGG CAGGCTGAGC	2040
	CTCCAGCTGC TCGTGGAGTG AGCTGGATGG CCGGCTCTTG ACAGCTATCT GAGAGGGGCG	2100
	CAGAACCAAA GGTGCCAGGG CTGAGGAGCT CAGGGGAGAG TGGTGGAGCT GAGGCTGTGG	2160
	GGAGGGAGCG CACAGGAGAG ACTGGTCTGT GTGTGGTCTG GCTTGGCTTC AGCTGAGTAA	2220
	GAGAGGGGCT CCGGCCCCCA GAGAACTTT AGGGCTAGCG CAGGCTCTTC AGCTACCTCA	2280
30	GCGCTGGGCT CCGGGGTTTA GGTAGGAGAG GTCCAGCTG CAGGCTCTTC CAGGAGGAG	2340
	AGAAGGTGTC TGTAGATTT AGGCTGGGGA CCGGAGTGA GAGACAGAGA AGCTAGGTTT	2400
	TGAGGAGACA GGGAGGAGAG CTTAGTGTAT CCGGCTTGA GCGAGGCTCA GAGGAGGCTC	2460
	CTGCTGGGCG CTGTAGGTTG GGTGAGGAGG GAGTGTAGC TACAGGAGGT GTGCTTCTTT	2520
	GAGGCTGAGG GGTGAGGAGG AGCTTGGGCG TCAGGCTCCG GAGGCTCTTC CTGAGGAGG	2580
35	AGCTGAGAGC TGTATTTTCC TGTCTTTTGA GAGTGAAGTG ATTGAGAAAC AAGGACTCAA	2640
	GTGCTTCTTC TCGAGGAGCA GGTGTCTCTC TCGCTGAATC ACTACGCTTC CCGCTACAC	2700
	TCTAGGTTTG GAGAGGAGCG TCTTGTCTTC CCGGCTTCA GCGTGGGCTT CCGCTGAGAA	2760
	TGTAGGAGAG ACAGGGAGAG CAGAGGAGTG GTGAGAGGAG AGGTCTCTTC GCGCGGGGAG	2820
	GGGGCGGGGT TCGGCTTTCG GAGAGGAGAG ACAGGAGTCA GTGAGGGGCG AGATTGAGAG	2880
40	ATCCCTCCCA CTCCCATCTC TGGGGCTTTC GGTGTCTTCA CCGGCTCTTC GCTCCGCTTC	2940
	CTCCCCAGGC CTACTTCTTC CTGAGGTTGA TCTGAGGAGG CAGGCTCTTC CCGCTGTGCG	3000
	CGCGCTCTAC CAGCGGCCCC CCGGAGTTTC AGCGGAGTCA CAGGCTCTTC TGAGGCGGCG	3060
	CGGAGGGGCG CCGGCGGGGG AGCGGAGTTC AGCGGAGTTC CAGGCTCTTC AGCTTGGAGG	3120
	CCGCGGCGCT CATCCGAGCC GCTTACGAGC GAGTGAAGTG CAGGCTCTTC TCGGCTGTTC	3180
45	TCTCTGCTTC GCTCTGGGTC CCGGCTCTTC TCTTCTCTTC AGCTTGGGCT GTGGGGAGG	3240
	GGGCTAGGCG CTGGAGCTTC GCGAGGCGCG CAGGCTCTTC ACAGGCTCTTC TCGTGTCTTC	3300
	TGCGAGGGGC GCGGCGCTTC TCGGCTCTTC TCTTCTCTTC CAGGCTCTTC CCGTACTTTC	3360

-3-

AGGCTACCC GCGCTCGCG CAGCTCAGGT GAGGACCGG CCGGABKCG GCGGGGCCG 3420
 GAGAAABATC GCGGCGCGG GGGGCTCCTG GGGGACGGGA CCGAAGCTCG GCGGABKCG 3480
 CCGGCGCGG CCGGCGCGG TTGGGATTC GGTGGGCGAG CCTGGCGGC GCGGABKCG 3540
 AGTCCCCGTG GCGGCGCGG TCGGCGCGG AAGAAGCGG CCGCTCGCG CCGGABKCG 3600
 5 GGTGACCGCC GCGGCGCGG TCGGCGCGG GTAGGCGAG GCGGCGCGG TCGGCGCGG 3660
 GGTGGCGCGG GGTGGCGCGG GGTGGCGCGG GGTGGCGCGG GGTGGCGCGG GGTGGCGCGG 3720
 GGTGGCGCGG GGTGGCGCGG GGTGGCGCGG GGTGGCGCGG GGTGGCGCGG GGTGGCGCGG 3780
 GGTGGCGCGG GGTGGCGCGG GGTGGCGCGG GGTGGCGCGG GGTGGCGCGG GGTGGCGCGG 3840
 GGTGGCGCGG GGTGGCGCGG GGTGGCGCGG GGTGGCGCGG GGTGGCGCGG GGTGGCGCGG 3900
 10 GGTGGCGCGG GGTGGCGCGG GGTGGCGCGG GGTGGCGCGG GGTGGCGCGG GGTGGCGCGG 3960
 GGTGGCGCGG GGTGGCGCGG GGTGGCGCGG GGTGGCGCGG GGTGGCGCGG GGTGGCGCGG 4020
 GGTGGCGCGG GGTGGCGCGG GGTGGCGCGG GGTGGCGCGG GGTGGCGCGG GGTGGCGCGG 4080
 GGTGGCGCGG GGTGGCGCGG GGTGGCGCGG GGTGGCGCGG GGTGGCGCGG GGTGGCGCGG 4140
 GGTGGCGCGG GGTGGCGCGG GGTGGCGCGG GGTGGCGCGG GGTGGCGCGG GGTGGCGCGG 4200
 15 GGTGGCGCGG GGTGGCGCGG GGTGGCGCGG GGTGGCGCGG GGTGGCGCGG GGTGGCGCGG 4260
 GGTGGCGCGG GGTGGCGCGG GGTGGCGCGG GGTGGCGCGG GGTGGCGCGG GGTGGCGCGG 4320
 GGTGGCGCGG GGTGGCGCGG GGTGGCGCGG GGTGGCGCGG GGTGGCGCGG GGTGGCGCGG 4380
 GGTGGCGCGG GGTGGCGCGG GGTGGCGCGG GGTGGCGCGG GGTGGCGCGG GGTGGCGCGG 4440
 GGTGGCGCGG GGTGGCGCGG GGTGGCGCGG GGTGGCGCGG GGTGGCGCGG GGTGGCGCGG 4465
 20

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

- 25 (A) LENGTH: 30 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

GCGGCGCGG GCGGCGCGG AGGCGGGGCA

30

(2) INFORMATION FOR SEQ ID NO: 3:

35

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 40 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

45 GTTGGACAGG TTCTCTCTG GCGGCGCGG

30

(2) INFORMATION FOR SEQ ID NO: 4:

-4-

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

GGGTTCGAC GACACGCTG CATTGACAC

30

(2) INFORMATION FOR SEQ ID NO: 5:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

ATGCTCCAC GCGAGCTCT GCGAGGAGT

30

(2) INFORMATION FOR SEQ ID NO: 6:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

CTCTGCTGTT GATTCTGGT TATCTCCCTT

30

(2) INFORMATION FOR SEQ ID NO: 7:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

-5-

{xi} SEQUENCE DESCRIPTION: SEQ ID NO: 7:

TAGTCGTTGT AGGGGTTCGA TGCACAGGT

30

5 (2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

{xi} SEQUENCE DESCRIPTION: SEQ ID NO: 8:

TAATCTCTCC ACCACCTTA TGTGTTCCG

30

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

{xi} SEQUENCE DESCRIPTION: SEQ ID NO: 9:

GACCAACAC ACACCACTCC TGGCTGTCCA

30

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

{xi} SEQUENCE DESCRIPTION: SEQ ID NO: 10:

GCACTTCTCG CTGTCTCCAT C

21

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 base pairs

-6-

- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

- 5 (ii) MOLECULE TYPE: other nucleic acid
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

GGAGTTCTGG GTGTCTCCAT T

21

- 10 (2) INFORMATION FOR SEQ ID NO: 12:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 21 base pairs
 - (B) TYPE: nucleic acid
 - 15 (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: other nucleic acid
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

20

TAGGGGTTCC ATGCACAAGG G

21

- (2) INFORMATION FOR SEQ ID NO: 13:

- 25 (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 21 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

30

- (ii) MOLECULE TYPE: other nucleic acid
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

TAGGGGTTCC ATGCACAATG G

21

35

- (2) INFORMATION FOR SEQ ID NO: 14:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 21 base pairs
 - 40 (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: other nucleic acid
- 45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

TAGGGGTTCC ATGCACAAGC T

21

-7-

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

- 10 (ii) MOLECULE TYPE: other nucleic acid
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

TAGGGGTTCC ATGCACATG T

21

15 (2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:

- 20 (A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: other nucleic acid
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

25 CACCCACCTT ATCTGTTCC T

21

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:

- 30 (A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

- 35 (ii) MOLECULE TYPE: other nucleic acid
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

CACCCACCTT ATCTGTTCC G

21

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:

- 45 (A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

-8-

- (i) MOLECULE TYPE: other nucleic acid
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

5 GGAACAGCCT GGATGGGTC A

21

(2) INFORMATION FOR SEQ ID NO: 19:

- (i) SEQUENCE CHARACTERISTICS:
10 (A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: other nucleic acid
15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

GGAACAGCCT GGATGGGTC C

21

20 (2) INFORMATION FOR SEQ ID NO: 20:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
25 (C) STRANDEDNESS: single
(D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: other nucleic acid
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

30

CAGACCAGTG CTGGCTGTAC G

21

(2) INFORMATION FOR SEQ ID NO: 21:

- (i) SEQUENCE CHARACTERISTICS:
35 (A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

40

- (ii) MOLECULE TYPE: other nucleic acid
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

CAGACCAGTG CTGGCTGTAC A

21

45

(2) INFORMATION FOR SEQ ID NO: 22:

-9-

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

10 GGTCACTCTG GACTTTGCCA C

21

(2) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

20 TTGTACCTT GAGGCAAGAG G

21

25 (2) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

35 AGGCTGGCAG GCATGAGTT T

21

(2) INFORMATION FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

TTCTGTGCCCC TTCTTTGCT A

21

(2) INFORMATION FOR SEQ ID NO: 26:

5

(1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

10

(D) TOPOLOGY: linear

{ii} MOLECULE TYPE: other nucleic acid

{xi} SEQUENCE DESCRIPTION: SEQ ID NO: 26:

15 CTCCAGCTGC TCTGCACTG A

21